Attorney Docket No: 0152.00384

## **REMARKS**

The above amendment added no new matter and is merely made to more accurately describe and claim the invention.

It is respectfully submitted that the application is now in condition for allowance, which allowance is respectfully requested.

The Commissioner is authorized to charge any fee or credit any overpayment in connection with this communication to our Deposit Account No. 11-1449.

Respectfully submitted,

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Dated: April 24, 2002

**CERTIFICATE OF MAILING** 

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.Ø. 20231 on April 24, 2002.

Connie Herty

Attorney Docket No: 0152.00384

**VERSION WITH MARKINGS TO SHOW CHANGES MADE** 

**SPECIFICATION:** 

Page 9, Lines 36-33:

Figure 3 is a photograph demonstrating the specificity of rabbit antiserum to

ebaf by Western blot analysis; in each lane, 10µg of extracted endometrial proteins

was resolved in a 15% gel by SDS-PAGE and then subjected to Western blot

analysis; the blot was probed with the antiserum alone (left lane) and with the

antiserum-preincubated with a 100 molar excess if the CASDGALVPRRLQHRP-

amide (Seq. ID. No. 3);

Page 16, Lines 1-7:

lane 1: molecular weight markers. 75 µg of placental proteins (lane 2), and cytosolic

proteins of late proliferative (lanes 3-4) and the late secretory (lanes 5-7) endometria

were subjected to Western blot analysis using the affinity purified rabbit antiserum

against a peptide (CASDGALVPRRLQHRP-amide) (Seq. ID. No. 3) at the C terminal

domain of the ebaf;

Page 17, Lines 1-6:

rabbit anti-serum to ebaf by Western blot analysis; A: in each lane, 10 micrograms of

extracted endometrial proteins was resolved in a 15% gel by SDS-PAGE and then

subjected to Western blot analysis; the blot was probed with the anti-serum alone

(left lane) and with the antiserum-preincubated with a 100 molar excess of the

CASDGALVPRRLQHRP-amide (Seq. ID. No. 3);

Page 27, Lines 20-27:

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One additional embodiment of the present invention is the development of an

antisera for ebaf. An antibody with specificity is useful in determining the presence

of ebaf, or an ebaf variant, in a sample. By variant, it is meant that an variant which

is functionally relevant. Further, the peptide CASDGALVPRRLQHRP-amide (Seq.

ID. No. 3), as demonstrated in the examples below, has been shown to be effective

in the development of such an antisera.

Page 47, Lines 11-22:

The polyclonal rabbit antibody raised against a synthetic peptide at the C

terminal domain of the ebaf reacted with a major 41 kDa protein in the placenta as

well as the endometrium. In the case of lefty, which is the mouse homologue of the

human ebaf, the expression of the protein in 293T cells led to formation of a non-

secretory, 42 kDa protein which is the size of the pre-pro-protein (Meno et al, 1996).

The predicted size of the pre-pro-protein of the ebaf is 41 kDa. The members of the

TGF-β super family are synthesized as pre-pro-proteins which are cleaved at RXXR

(Seq. ID. No. 2) sites to release the mature form of the protein. The predicted protein of ebaf exhibits two such RXXR sites (Seq. ID. No. 2) which are located at

amino acid residues of 73-76 and 131-134 respectively (Kothapalli et al, 1997).

Page 48, Lines 1-2:

to cleavage at the first and second RXXR (Seq. ID. No. 2) sites respectively

(Kothapalli et al, 1997).

Page 55, Lines 1-4:

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expected to be secreted (Table 2). To detect such proteins in human endometrium, an antiserum was raised against the peptide CASDGALVPRRLQHRP-amide (Seq. ID. No. 3) at the COOH terminal of the ebaf protein.

Page 62, Lines 14-21:

PCR was carried out as described using the 5' primer (B2P9): TCAGCGAGGTGCCCGTACT (Seq. ID. No. 4) and 3' primer (B2P1): AGTTCTTAGAGCTGAAGCC (Seq. ID. No. 5). Briefly, 1  $\mu$ g of reverse transcribed RNA was amplified with 0.5-1  $\mu$ M of each of the 5' and 3' primers specific for *ebaf* in a 50  $\mu$ l reaction volume containing 1.25 U AmpliTaq DNA polymerase, 1.25 mM MgCl<sub>2</sub>, 20  $\mu$ M of each of dATP, dCTP, dGTP, dTTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and sterile distilled water.

Page 65, Lines 22-26:

Premature Expression Of ebaf protein In The Endometria Of Infertile Patients: To localize the ebaf protein in endometrium, two polyclonal rabbit antisera were raised against a sequence (CASDGALVPRRLQHRP) (Seq. ID. No. 3) that resides at the carboxy terminal end of the express *ebaf*.

Page 71, Lines 8-13:

A monoclonal and rabbit antisera were raised against the peptide CASDGALVPRRLQHRP-amide (Seq. ID. No. 3) at the COOH terminal (Tabibzadeh et al, 1998) and to acetyl-DRADMEKLVIPAC peptide (Seq. ID. No. 6) at the NH2 terminal of the *ebaf* (Figures 25-26). Rabbit antiserum to CASDGALVPR RLQHRP-amide (Seq. ID. No. 3) was purified on a peptide column.

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Please insert Sequence Listing after Page 81, after Table 7, of the Specification.